Fractionation of Adipose Tissue Procedure With a Disposable One-Hole Fractionator

van Dongen, Joris A; Gostelie, Olivier F E; Vonk, Lucienne A; de Bruijn, Julia J; van der Lei, Berend; Harmsen, Martin C; Stevens, Hieronymus P

Published in:
Aesthetic Surgery Journal

DOI:
10.1093/asj/sjz223

Publication date:
2020

Fractionation of Adipose Tissue Procedure With a Disposable One-Hole Fractionator

Joris A. van Dongen, BSc; Olivier F. E. Gostelie, MD; Lucienne A. Vonk, PhD; Julia J. de Bruijn, BSc; Berend van der Lei, MD, PhD; Martin C. Harmsen, PhD; and Hieronymus P. Stevens, MD, PhD

Mr van Dongen and Ms de Bruijn are Students, Department of Pathology and Medical Biology, University of Groningen and University Medical Center of Groningen, Groningen, the Netherlands. Dr Gostelie is a Resident, Department of Plastic Surgery, Maasstad Hospital, Rotterdam, the Netherlands. Dr Vonk is an Assistant Professor, Department of Orthopedics, University Medical Center Utrecht, Utrecht, the Netherlands. Dr van der Lei is a Professor, Department of Plastic Surgery, University of Groningen and University Medical Center of Groningen, Groningen, the Netherlands. Dr Harmsen is a Professor of Cardiovascular Regenerative Medicine, Department of Pathology and Medical Biology, University of Groningen and University Medical Center of Groningen, Groningen, the Netherlands. Dr Stevens is a Plastic Surgeon, Department of Plastic Surgery, Velthuis Kliniek, Rotterdam, the Netherlands.

Corresponding Author: Dr. Hieronymus P. Stevens, Velthuis Kliniek Rotterdam, K.P. Mandelelaan 10, 3062 MB Rotterdam
E-mail: stevens.hp@gmail.com; Twitter: @dokterstevens

Disclosures: The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding: The authors received no financial support for the research, authorship, and publication of this article.

Abstract

Background: Adipose tissue has been widely used in regenerative surgery for its therapeutic potential. The therapeutic potential of adipose tissue is often ascribed to the stromal vascular fraction (SVF), which can be mechanically isolated. Mechanical isolation results in SVF containing intact cell-cell communication including extracellular matrix and is therefore named tissue-SVF (tSVF).

Objectives: The fractionation of adipose tissue (FAT) procedure, a procedure to mechanically isolate tSVF, is evaluated using a new disposable one-hole fractionator and compared to the former reusable three-hole fractionator.

Methods: The composition of tSVF obtained by the one-hole fractionator was histologically and histochemically compared to unprocessed adipose tissue. The number of viable nuclear cells in tSVF obtained by the onehole and three-hole fractionator as well as unprocessed adipose tissue were compared after enzymatic isolation and tested for colony forming unit capacity. Flow cytometry was used to compare different cell compositions based on surface marker expression between tSVF isolated by both types of fractionators.

Results: Fractionation of adipose tissue with the one-hole fractionator condenses vasculature and extracellular matrix by disrupting adipocytes. The number of viable nuclear cells in tSVF obtained by both fractionators was comparable and significantly higher than unprocessed lipoaspirate. Furthermore, tSVF isolated by both types of fractionators showed similar cell compositions and comparable colony forming unit capacities.

Conclusions: The FAT procedure with a disposable one-hole fractionator effectively isolates tSVF with a comparable cell count and cell composition in comparison with the three-hole reusable fractionator. A disposable one-hole fractionator, however, is safer and more user friendly.

Adipose tissue has been widely used in regenerative- and reconstructive surgery for its therapeutic potential in the treatment of osteoarthritis, as anti-scarring treatment, to improve wound healing and to compensate for volume loss.[1-4] The therapeutic potential of adipose tissue is often ascribed to the presence of adipose tissue-derived stromal cells (ASCs) in stromal vascular fraction (SVF).[5] SVF of adipose tissue consist of all non-parenchyma, ie, non-adipocyte cell types such as ASCs, fibroblasts, vascular cells, immune cells as well as extracellular matrix (ECM).[6-10] Upon culturing, ASCs develop from their *in vivo* precursors, ie, pericytes or supra-adventitial cells.[11,12] Cultured ASCs secrete a plethora of growth factors and exosomes that are pro-regenerative and stimulate angiogenesis, promote proliferation of parenchymal cells, suppress apoptosis and modulate immune responses in vivo.[1,13,14] In addition, ASCs secrete ECM components as well as ECM degrading enzymes, which are relevant for tissue remodeling. ASCs are readily isolated from lipoaspirates in high numbers by both enzymatic and mechanical procedures.[15] However, enzymatic isolation is rather costly and time-consuming, because of reagents used such as collagenase that are of non-human origin.[14] Enzymatic isolation procedures result in a single cell suspension of SVF (cSVF) that lacks cell-cell and cell-matrix connections, whereas mechanical isolation procedures result in a tissue-like SVF (tSVF) containing ECM as well as all cell-cell and cell-matrix connections.[15,16] In tSVF, ECM functions as a scaffold for SVF cells and prevents cells from diffusion after injection and therefore might enhances tissue regeneration. Additionally, ECM functions as a slow release reservoir of growth factors.[8,9,17] The interaction between cells and growth factors in tSVF is postulated to increase the regenerative potential as compared to the use of cSVF.

One of the recently published mechanical isolation procedures is the 'Fractionation of Adipose Tissue' (FAT) procedure, which is a fast and low cost intraoperative procedure to obtain tSVF from adipose tissue.[18] It appeared that the original FAT device and procedure would benefit from improvements for several reasons. Firstly, the original fractionator contains an internal disk with three off-centered holes of 1.4 mm. In our experience, the absence of a central hole in the original fractionator leads to congestion in the device, which makes the FAT procedure less practical. Secondly, the original fractionator is a non-disposable device. In view of patient safety, however, sterile disposable devices are preferred over non-disposable devices. For all the aforementioned reasons, a new disposable one-hole fractionator was developed. The purpose of this study is to compare the one-hole fractionator with the original reusable three-hole fractionator based on the composition of the isolated tSVF.

METHODS

Liposuction and FAT Procedures

Liposuction as well as the FAT procedure were executed as described in the study of van Dongen et al between September 2017-September 2018.[18-20] All included patients gave informed consent according to the Declaration of Helsinki. In short, adipose tissue harvesting was performed with a Sorenson lipoharvesting cannula (Tulip, Medical Products, San Diego, CA) during normal liposuction procedures in three patients. Lipoaspirate was centrifuged after decantation and mechanically dissociated either by the three-hole fractionator or the one-hole fractionator (Tulip, Medical Products, San Diego, CA) (Supplemental Figure 1, available online as Supplementary Material at www.aestheticsurgeryjournal.com). After mechanical dissociation, samples from both types of fractionators were centrifuged again yielding a comparable amount of oil (8.4 ml ± 0.4), tSVF (1.1 ml ± 0.4) and infiltration fluid fraction containing a pellet (0.5 ml) (Supplemental Figure 1). One sample of centrifuged lipoaspirate of each patient (n=3) was used as a control.

Immunohistochemistry and Masson's Trichrome

Samples of tSVF obtained by the one-hole fractionator and control lipoaspirate (n=3) were fixed in 10% formalin in phosphate buffered saline (PBS), embedded in paraffin and 4 µm sections were cut. Immunohistochemistry, ie, α-Smooth Muscle Actin (SMA) and Perilipin, as well as Masson's Trichrome staining were performed according to the protocol of van Dongen et al.[16] Primary antibodies used in this study were α-SMA (1:200, Abcam, Cambridge, UK) and Perilipin (1:200, Abcam, Cambridge, UK). Secondary antibodies used were polyclonal Rabbit anti-Mouse for α-SMA (1:100, DAKO, Glostrup, Denmark) as well as polyclonal Goat anti-Rabbit for Perilipin (1:100, DAKO, Glostrup, Denmark). A tertiary antibody was only used for α-SMA (polyclonal Swine anti-Rabbit for 1:100, DAKO, Glostrup, Denmark). All samples were visualized and evaluated by light microscopy (Leica Microsystems, DM IL).

Cell Isolation and Culture

Samples of tSVF obtained by the one-hole fractionator and the three-hole fractionator as well as control lipoaspirate (n=3) were enzymatically dissociated in 0.1% collagenase A, 1%

bovine serum albumin in PBS and cultured according to our previously published protocol.[16] Cells were counted upon staining with trypan-blue in a Bürker Türk counting chamber.

Flow Cytometry

Cells collected from enzymatically dissociated tSVF samples, ie, the one-hole fractionator and the three-hole fractionator (n=3) were analyzed for surface marker expression using flow cytometry. Cells were labelled with the following anti-human monoclonal antibodies: CD31, CD34, CD90, CD105, CD146 (Miltenyi Biotec Bergisch Gladbach, Germany) and CD45 (Biolegend, San Diego, CA, USA) as well as 7-Amino Actinomycin D (Invitrogen, molecular probes, Eugene, OR, USA) to stain for dead cells. Cells were mixed well with the antibodies in FACS buffer (5 mM ethylenediaminetetraacetid acid (EDTA), 1% BSA in PBS) and incubated on ice and in dark for 30 min. Stainings with a single antibody and fluorescence minus one (FMO) were used as controls. A BD FACSCanto II system (BD Biosciences) was used to analyze the samples.

Colony Formation Assay

Ten thousand viable cells isolated from tSVF obtained by the one-hole fractionator and the three-hole fractionator (n=3) were seeded (six technical replicates) and cultured for twelve days to assess the colony forming capacity of uncultured cells from a single cell. Afterwards, cells were fixed in 4% formalin and stained with 5% Crystal Violet (Sigma-Aldrich, St. Louis, MO). Colony frequency was calculated as the mean number of colonies / total seeded cells x 100%.

Statistical Analysis

Immunohistochemistry staining were analyzed with the use of ImageJ, version 1.4.3.67 (NIH, USA).[17] Descriptive statistics were used to evaluate α-Smooth Muscle Actin (SMA), cell numbers with the use of Graphpad Prism, version 5.01 (Graph Pad Software Inc., Los Angeles). Data were expressed as mean ± standard deviation (SD). A paired *t*-test was performed with the use of Graphpad Prism, version 5.01 (Graph Pad Software Inc., Los Angeles).

RESULTS

Fractionation of Adipose Tissue Obtained by the One-Hole Fractionator Condenses tSVF

More α-smooth muscle actin (α-SMA) expression was observed in tSVF (0.83% ± 0.33) as compared to control lipoaspirate (0.094% ± 0.036) for ($p<0.05$), indicating a higher number of small vessels in tSVF obtained by the one-hole fractionator (Figure 1). Control lipoaspirate was rich in adipocytes (visually analyzed based on perilipin A expression) while tSVF was essentially devoid of adipocytes. This difference indicates that adipocytes were destructed by the fractionator. More collagen was present in tSVF in comparison to control lipoaspirate, which indicates that the fractionation condensed ECM.

Fractionation of Adipose Tissue Obtained With Either the Three-Hole Fractionator or the One-Hole Fractionator Condenses Cells

Enzymatic isolation of tSVF obtained by the one-hole fractionator and the three-hole fractionator as well as control lipoaspirate resulted in adipocyte-free cell preparation with a mean viable nuclear cell count of respectively $2.35*10^6 \pm 2.98*10^5$, $2.67*10^6 \pm 4.63*10^5$ and $3.12*10^5 \pm 8.99*10^4$ (Figure 2). No quantitative differences were found for ASCs (CD45-; CD90+; CD105+: reusable 41.4% ± 16.5%, disposable 44.9% ± 18.2%), endothelial cells (CD31+; CD34+: reusable 12.0% ± 4.5%, disposable 19.1% ± 2.3%), leukocytes (CD45+; CD34-: reusable 5.3% ± 3.6%, disposable 5.3% ± 3.6%), pericytes (CD34+/-; CD31-; CD146+: reusable 0.3% ± 0.3%, disposable 0.5% ± 0.5%) and hematopoietic stem cell-like cells (CD45+; CD34+: reusable 0.1% ± 0.2%, disposable 0.2% ± 0.3%) after the FAT procedure with either the three-hole fractionator or the one-hole fractionator (Figure 2). The number of supra-adventitial cells (CD34bright; CD31-, CD146-) was uncountable low in both types of tSVF. Thus, fractionation by means of the one-hole fractionator as well as the three-hole fractionator reduced the volume of lipoaspirates while the stromal-vascular cells were condensed as compared to control lipoaspirate. No difference in cell count in tSVF was seen between both fractionation procedures.

Fractionation of Adipose Tissue Does Not Affect Colony Formation

Colony frequency of uncultured cells derived from tSVF by means of the one-hole fractionator and the three-hole fractionator was similar with respectively 1.29% ± 0.038 and 1.29% ± 0.045 ($p>0.05$) (Figure 3).

DISCUSSION

The FAT procedure with the use of the one-hole disposable fractionator showed to be as effective to isolate tSVF with a comparable number of cells and composition of cell types as

compared to the former FAT procedure. In our experience, this new system never blocked upon use. The obtained tSVF by means of the one-hole disposable fractionator was composed of condensed vasculature and ECM in comparison with unprocessed adipose tissue. After enzymatic digestion of the tSVF obtained by both types of fractionators, cells were able to attach to tissue culture plastic and form colonies, indicating the colony forming function has not been disturbed by both systems. However, only low number of donors were used for experiments which might causes a larger standard deviation of the results and is thus a limitation of this study.

Characterization of the composition of cell types in the isolated tSVF can be done by surface marker analysis. In adipose tissue, cells can be divided into two major subpopulations: adipose derived cells (CD45-) and blood derived cells (CD45+).[21] Adipose derived cell populations can be further divided into two main subpopulations: endothelial like cell types (CD31+) and stromal like cell types (CD31-).[21] Three important cell types within the stromal like cell subpopulation (CD45-; CD31-) are the ASCs (CD34+; CD90+; CD105low) and ASCs precursor cells: pericytes (CD34+/-; CD146+) and supra-adventitial cells (CD34+; CD146-).[11,21-23] However, controversy remains about the ASCs precursor cell types and CD surface marker combinations to identify different adipose tissue cell subpopulations.[11,12,23,24] ASCs and its precursor cells are important cell types because of the secretion of many regenerative growth factors and cytokines.[1,13,14] In comparison to several other mechanical and enzymatic isolation procedures (eg, Automated isolation system, CHA Biotech Station, Lipokit Medikhan System, PNC's Multistation, Fastem and Lipogems), both types of FAT procedures isolate more stromal cells.[25-28] Enzymatic isolation procedures (eg, Cytori, Tissue Genesis Cell Isolation System, Sepax System) showed comparable stromal cell populations.[26,29,30] However, differences in cell subpopulations of cSVF and tSVF could be caused by donor dependent variations and different use of CD surface markers and thus comparisons between studies are difficult.[18-21]

The use of the one-hole fractionator seems to offer several advantages over the non-disposable three-hole fractionator. Firstly, any re-usable device could bring a potential risk of contamination and biofilms to grow after sterilization, in particular in the difficult-to-clean small holes.[15,31] Using a disposable fractionator largely eliminates the risk of contamination and thus complies with the most restricted regulations on sterility. Yet, the potential increased risk of contamination in reusable devices in comparison with disposable devices is solely hypothetical instead of based on sterility data, which is another limitation of this study. To even further reduce the risk of contamination during the isolation procedure, a completely

closed system could be designed to promote safe clinical use (which has been manufactured in the meantime (ACA-kit, Arthex GmbH, USA). Secondly, in the one-hole fractionator, the opening in the internal disk is situated in the center of the disk resulting in significant fewer blockages. In the original three-hole fractionator, the internal disk contains three holes without a hole in the center of the disk. The tip of the 10 mL syringe faces the center of the three-hole disk in the middle of the fractionator. In this way, blockage of the three-hole fractionator can occur when lipoaspirate contains substantial amounts of fibrous tissue (eg, in secondary donor sites or in male subjects with limited subcutaneous tissue to harvest from).

Thus far, multiple *in vitro* studies have investigated the composition of the isolated tSVF by means of the FAT procedure or similar mechanical procedures like the Nanofat procedure.[16,18,32] These studies have shown that tSVF contains increased numbers of ASCs as compared to unprocessed lipoaspirates.[16,32] ASCs are important cell types with a high regenerative potential. As mentioned before, ASCs secrete a plethora of growth factors and cytokines which are able to stimulate important regenerative processes such as matrix remodeling and angiogenesis.[1,17,33] Hence, the obtained tSVF is promising for clinical regenerative purposes, such as scar remodeling and wound healing. However, its clinical effectiveness has not been proven yet as no well-designed randomized double-blind placebo-controlled prospective clinical trials investigating the regenerative potential of tSVF have been performed. Well-defined prospective trials with the use of mechanical isolated tSVF are therefore warranted. To date, our research group finished the inclusion of two randomized double-blind placebo-controlled clinical trials using tSVF isolated by means of the FAT procedure to improve scarring after breast reductions and to improve skin quality for the aging face. A safe and easy-to-use disposable system, such as the disposable one-hole fractionator as described in this study, might also pave the way towards a comparative study.

CONCLUSION

The FAT procedure with the one-hole disposable fractionator effectively isolates tSVF with a comparable number of cells and cell composition in comparison with the original described FAT procedure using a three-hole reusable fractionator. However, a disposable one-hole fractionator offers technical and financial advantages over the reusable three-hole fractionator, as it is safer and more user friendly.

Supplementary Material

This article contains supplementary material located online at www.aestheticsurgeryjournal.com.

Acknowledgement

Mr van Dongen and Dr Gostelie made an equal contribution to this work as co-first authors.

REFERENCES

1. Spiekman M, van Dongen JA, Willemsen JC, Hoppe DL, van der Lei B, Harmsen MC. The power of fat and its adipose-derived stromal cells: Emerging concepts for fibrotic scar treatment. *J Tissue Eng Regen Med*. 2017;11(11):3220-3235.
2. Brongo S, Nicoletti GF, La Padula S, Mele CM, D'Andrea F. Use of lipofilling for the treatment of severe burn outcomes. *Plast Reconstr Surg*. 2012;130(2):374e-376e.
3. Tanikawa DY, Aguena M, Bueno DF, Passos-Bueno MR, Alonso N. Fat grafts supplemented with adipose-derived stromal cells in the rehabilitation of patients with craniofacial microsomia. *Plast Reconstr Surg*. 2013;132(1):141-152.
4. Jo CH, Chai JW, Jeong EC, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: A 2-year follow-up study. *Am J Sports Med*. 2017;45(12):2774-2783.
5. Atalay S, Coruh A, Deniz K. Stromal vascular fraction improves deep partial thickness burn wound healing. *Burns*. 2014;40(7):1375-1383.
6. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: A joint statement of the international federation for adipose therapeutics and science (IFATS) and the international society for cellular therapy (ISCT). *Cytotherapy*. 2013;15(6):641-648.
7. Eto H, Suga H, Matsumoto D, et al. Characterization of structure and cellular components of aspirated and excised adipose tissue. *Plast Reconstr Surg*. 2009;124(4):1087-1097.
8. Yue B. Biology of the extracellular matrix: An overview. *J Glaucoma*. 2014;23(8 Suppl 1):S20-3.

9. Briquez PS, Hubbell JA, Martino MM. Extracellular matrix-inspired growth factor delivery systems for skin wound healing. *Adv Wound Care (New Rochelle)*. 2015;4(8):479-489.
10. Kuno S, Yoshimura K. Condensation of tissue and stem cells for fat grafting. *Clin Plast Surg*. 2015;42(2):191-197.
11. Corselli M, Chen CW, Sun B, Yap S, Rubin JP, Peault B. The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. *Stem Cells Dev*. 2012;21(8):1299-1308.
12. Lin G, Garcia M, Ning H, et al. Defining stem and progenitor cells within adipose tissue. *Stem Cells Dev*. 2008;17(6):1053-1063.
13. Hassan WU, Greiser U, Wang W. Role of adipose-derived stem cells in wound healing. *Wound Repair Regen*. 2014;22(3):313-325.
14. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng*. 2001;7(2):211-228.
15. van Dongen JA, Tuin AJ, Spiekman M, Jansma J, van der Lei B, Harmsen MC. Comparison of intraoperative procedures for isolation of clinical grade stromal vascular fraction for regenerative purposes: A systematic review. *J Tissue Eng Regen Med*. 2018;12(1):e261-e274.
16. van Dongen JA, Stevens HP, Harmsen MC, van der Lei B. Mechanical micronization of lipoaspirates: Squeeze and emulsification techniques. *Plast Reconstr Surg*. 2017;139(6):1369e-1370e.
17. Kapur SK, Katz AJ. Review of the adipose derived stem cell secretome. *Biochimie*. 2013;95(12):2222-2228.
18. van Dongen JA, Stevens HP, Parvizi M, van der Lei B, Harmsen MC. The fractionation of adipose tissue procedure to obtain stromal vascular fractions for regenerative purposes. *Wound Repair Regen*. 2016;24(6):994-1003.
19. Mashiko T, Wu SH, Feng J, et al. Mechanical micronization of lipoaspirates: Squeeze and emulsification techniques. *Plast Reconstr Surg*. 2017;139(1):79-90.
20. Stevens HP, Donners S, de Bruijn J. Introducing platelet-rich stroma: Platelet-rich plasma (PRP) and stromal vascular fraction (SVF) combined for the treatment of androgenetic alopecia. *Aesthet Surg J*. 2018;38(8):811-822.
21. Yoshimura K, Shigeura T, Matsumoto D, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol*. 2006;208(1):64-76.

22. Corselli M, Crisan M, Murray IR, et al. Identification of perivascular mesenchymal stromal/stem cells by flow cytometry. *Cytometry A*. 2013;83(8):714-720.
23. Zimmerlin L, Donnenberg VS, Pfeifer ME, et al. Stromal vascular progenitors in adult human adipose tissue. *Cytometry A*. 2010;77(1):22-30.
24. Traktuev DO, Merfeld-Clauss S, Li J, et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ Res*. 2008;102(1):77-85.
25. Bianchi F, Maioli M, Leonardi E, et al. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. *Cell Transplant*. 2013;22(11):2063-2077.
26. Aronowitz JA, Ellenhorn JD. Adipose stromal vascular fraction isolation: A head-to-head comparison of four commercial cell separation systems. *Plast Reconstr Surg*. 2013;132(6):932e-9e.
27. Domenis R, Lazzaro L, Calabrese S, et al. Adipose tissue derived stem cells: In vitro and in vivo analysis of a standard and three commercially available cell-assisted lipotransfer techniques. *Stem Cell Res Ther*. 2015;6:2.
28. SundarRaj S, Deshmukh A, Priya N, Krishnan VS, Cherat M, Majumdar AS. Development of a system and method for automated isolation of stromal vascular fraction from adipose tissue lipoaspirate. *Stem Cells Int*. 2015;2015:109353.
29. Doi K, Tanaka S, Iida H, et al. Stromal vascular fraction isolated from lipo-aspirates using an automated processing system: Bench and bed analysis. *J Tissue Eng Regen Med*. 2013;7(11):864-870.
30. Guven S, Karagianni M, Schwalbe M, et al. Validation of an automated procedure to isolate human adipose tissue-derived cells by using the sepax(R) technology. *Tissue Eng Part C Methods*. 2012;18(8):575-582.
31. Roberts CG. The role of biofilms in reprocessing medical devices. *Am J Infect Control*. 2013;41(5 Suppl):S77-80.
32. Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, Declercq H. Nanofat grafting: Basic research and clinical applications. *Plast Reconstr Surg*. 2013;132(4):1017-1026.

33. Song YH, Shon SH, Shan M, Stroock AD, Fischbach C. Adipose-derived stem cells increase angiogenesis through matrix metalloproteinase-dependent collagen remodeling. *Integr Biol (Camb)*. 2016;8(2):205-215.

Figure Legend

Figure 1. (A) Light microscope images of α-SMA of tSVF obtained by an one-hole disposable fractionator. (B) Light microscope images of α-SMA of control lipoaspirate. (C) Light microscope images of masson's trichrome of tSVF obtained by an one-hole disposable fractionator. (D) Light microscope images of masson's trichrome of control lipoaspirate. (E) Light microscope images of perilipin staining of tSVF obtained by an one-hole disposable fractionator. (F) Light microscope images of perilipin staining of control lipoaspirate. (G) Statistic data of expression of α-SMA in tSVF obtained by an one-hole disposable fractionator and control lipoaspirate. Results are presented as mean ± standard deviation. *Significant more small blood vessels were visible in tSVF as compared to control lipoaspirate ($p<0.05$). α-SMA = alpha-smooth muscle actin; tSVF = tissue stromal vascular fraction.

Figure 2. (A) Statistic data of number of viable nucleated cells per 1 mL of tSVF obtained by the reusable fractionator as well as disposable fractionator and control lipoaspirate. Results are presented as mean ± standard deviation. *tSVF obtained by the disposable fractionator contains significant more viable nucleated cells in 1 mL as compared to 1 mL of control ($p=0.011$). (B) Flow cytometric analysis of freshly mechanical isolated tSVF for viable cells (7AAD). (C) Flow cytometric analysis of freshly mechanical isolated tSVF for cell size (FSC-A). (D) Flow cytometric analysis of freshly mechanical isolated tSVF for CD31 expression. (E) Flow cytometric analysis of freshly mechanical isolated tSVF for CD34 expression. (F) Flow cytometric analysis of freshly mechanical isolated tSVF for CD45 expression. (G) Flow cytometric analysis of freshly mechanical isolated tSVF for CD90 expression. (H) Flow cytometric analysis of freshly mechanical isolated tSVF for CD105 expression. (I) Flow cytometric analysis of freshly mechanical isolated tSVF for CD146 expression. (J) Statistic data of percentage of different cell population in tSVF isolated by means of fractionation with the disposable as well as the reusable fractionator. Results are presented as mean ± standard deviation. No significant differences between all types of cell populations, ie, ASCs (CD45-; CD90+; CD105+), endothelial cells (CD34+; CD31+) and leukocytes (CD45+; CD34-). (K) Statistic data of percentage of different cell population in tSVF isolated by means of fractionation with the disposable as well as the reusable fractionator. Results are presented as mean ± standard deviation. No significant differences between all types of cell populations, ie, pericytes (CD34dim; CD31-; CD146+) and hematopoietic stem cell-like cells (CD45+; CD34+). The number of supra-adventitial cells (CD34bright; CD31-; CD146-) were uncountable low in tSVF isolated by the one-hole fractionator and the three-hole fractionator.

tSVF = tissue stromal vascular fraction; the one-hole fractionator = disposable fractionator; the three-hole fractionator = reusable fractionator. ASCs = adipose tissue-derived stromal cells.

Figure 3. Colony frequency of uncultured cells derived from tSVF isolated by the reusable fractionator as well as disposable fractionator. No significant difference between colony frequency of uncultured cells derived from tSVF. P0 = passage 0; tSVF = tissue stromal vascular fraction; the one-hole fractionator = disposable fractionator; the three-hole fractionator = reusable fractionator.

Figure 1A.

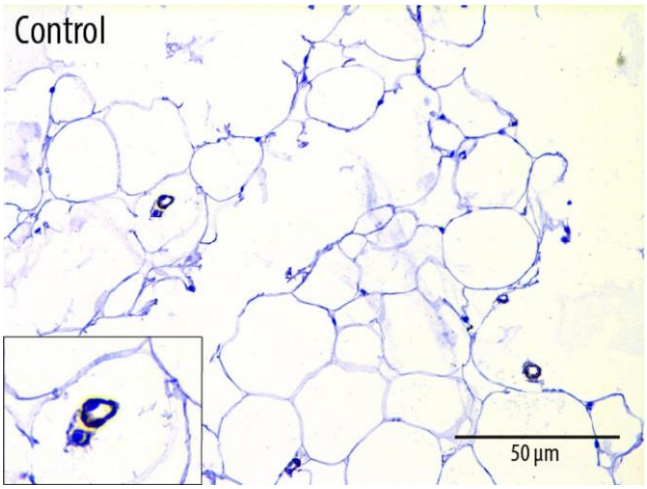

Figure 1B.

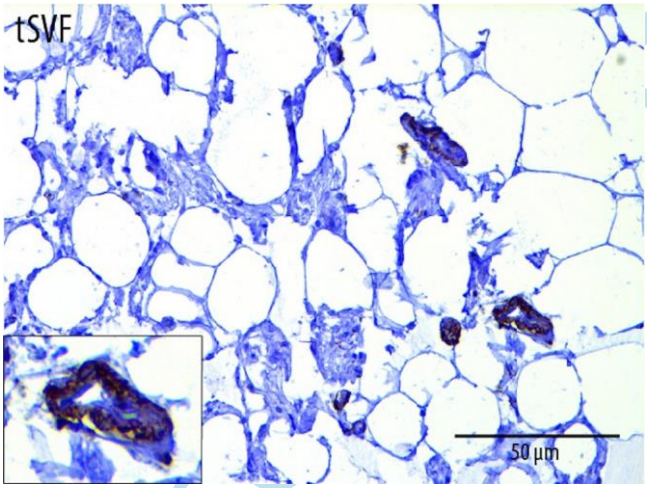

Figure 1C.

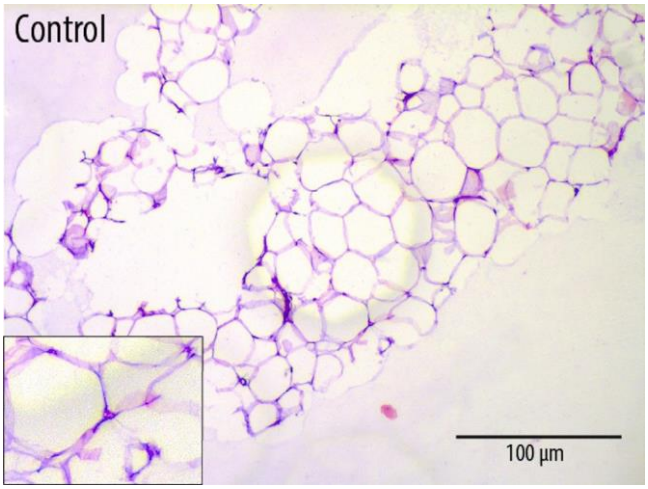

Figure 1D.

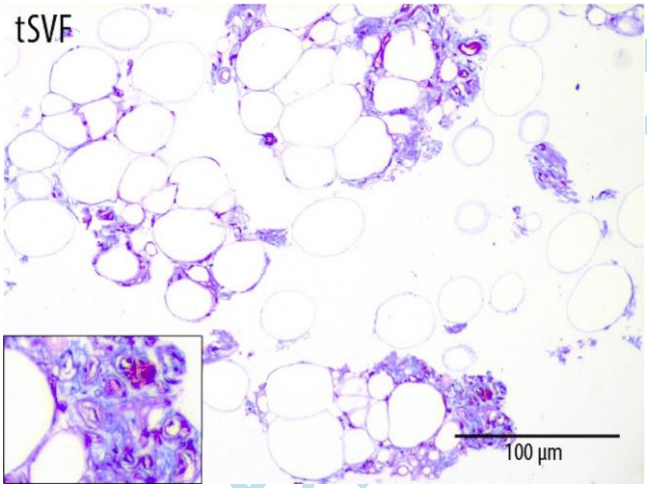

Figure 1E.

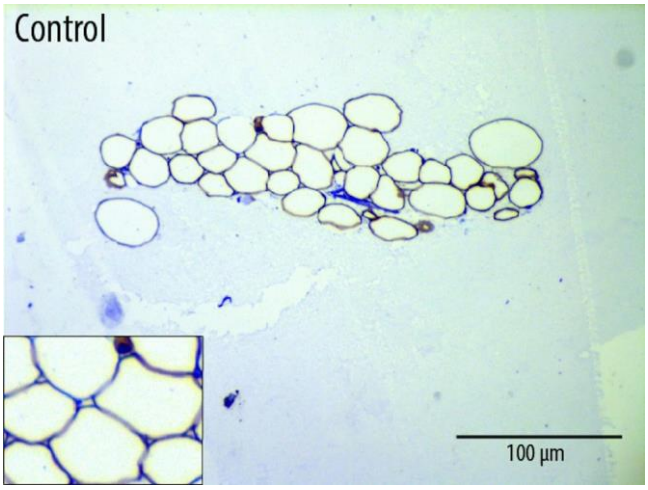

Figure 1F.

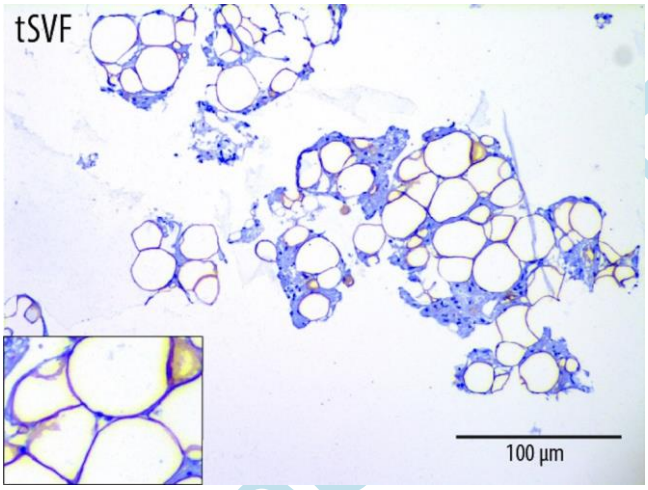

Figure 1G.

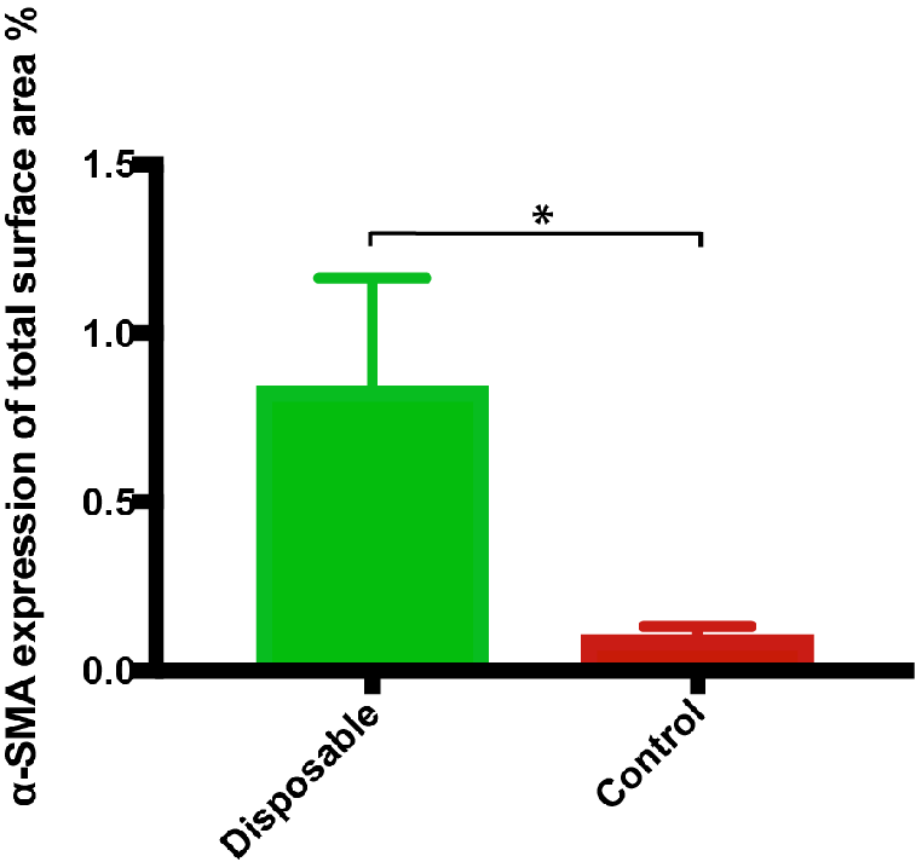

Figure 2A.

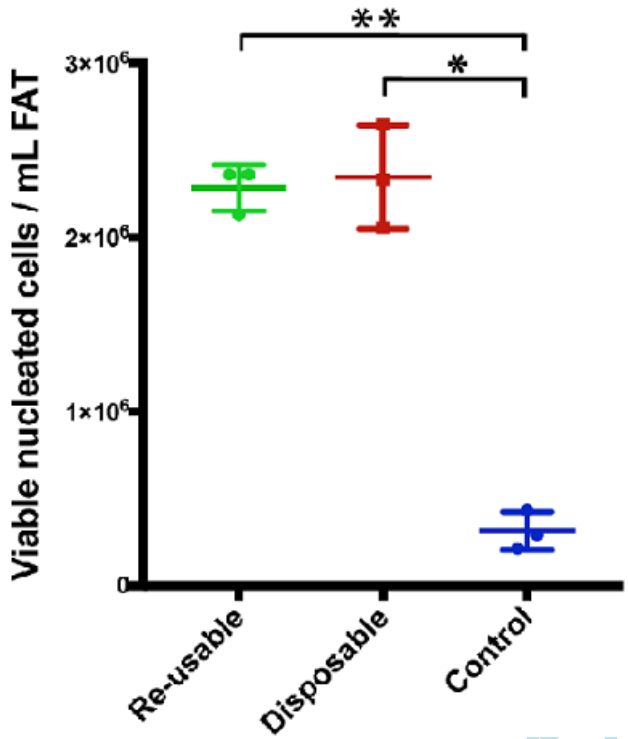

Figure 2B.

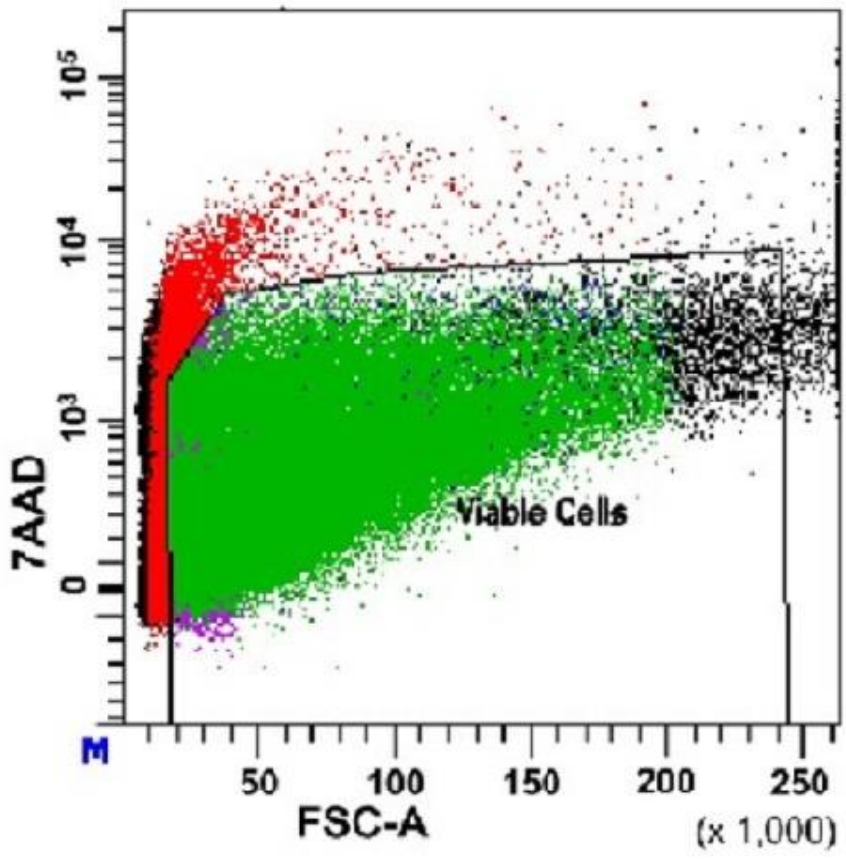

Figure 2C.

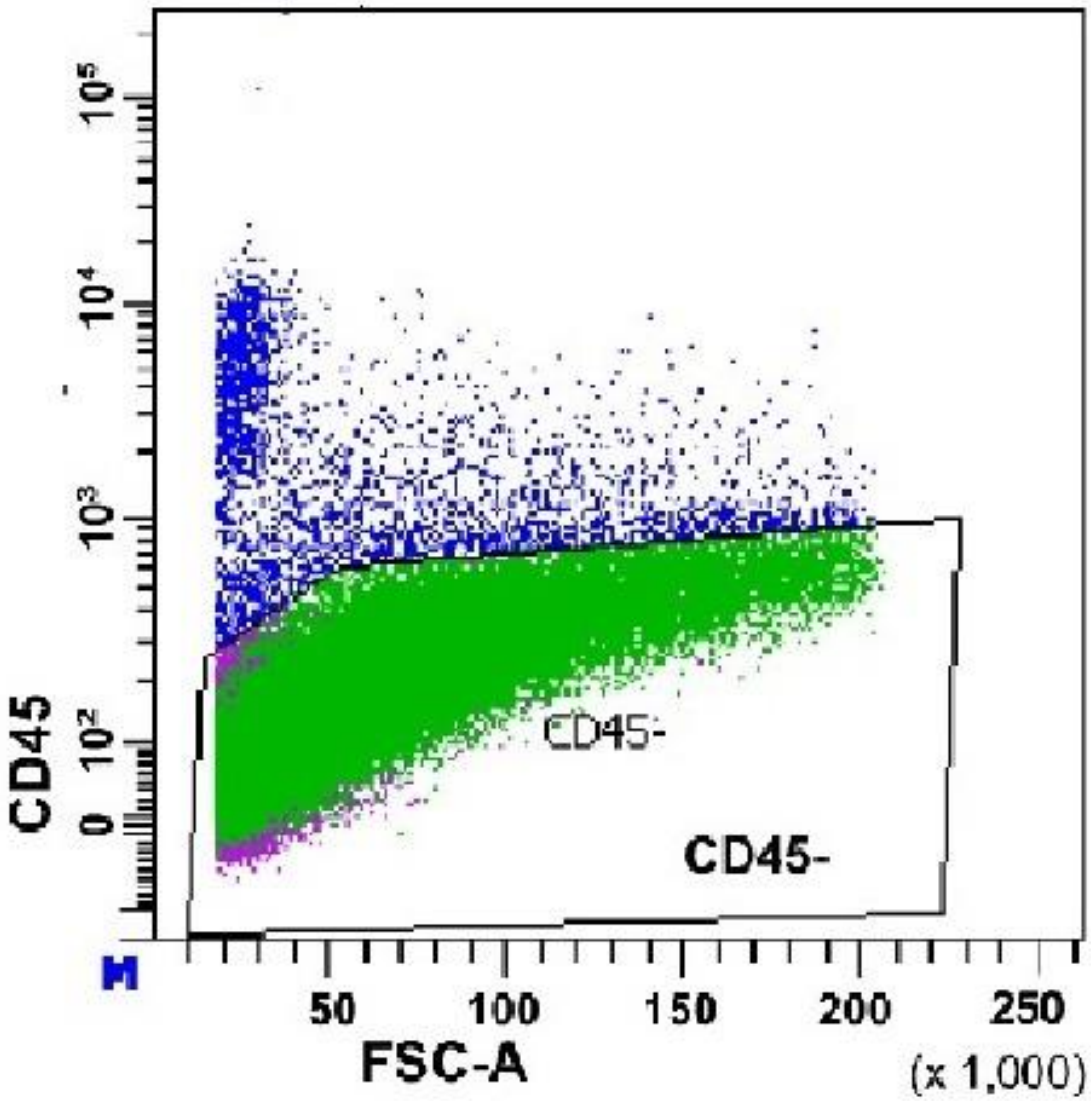

Figure 2D.

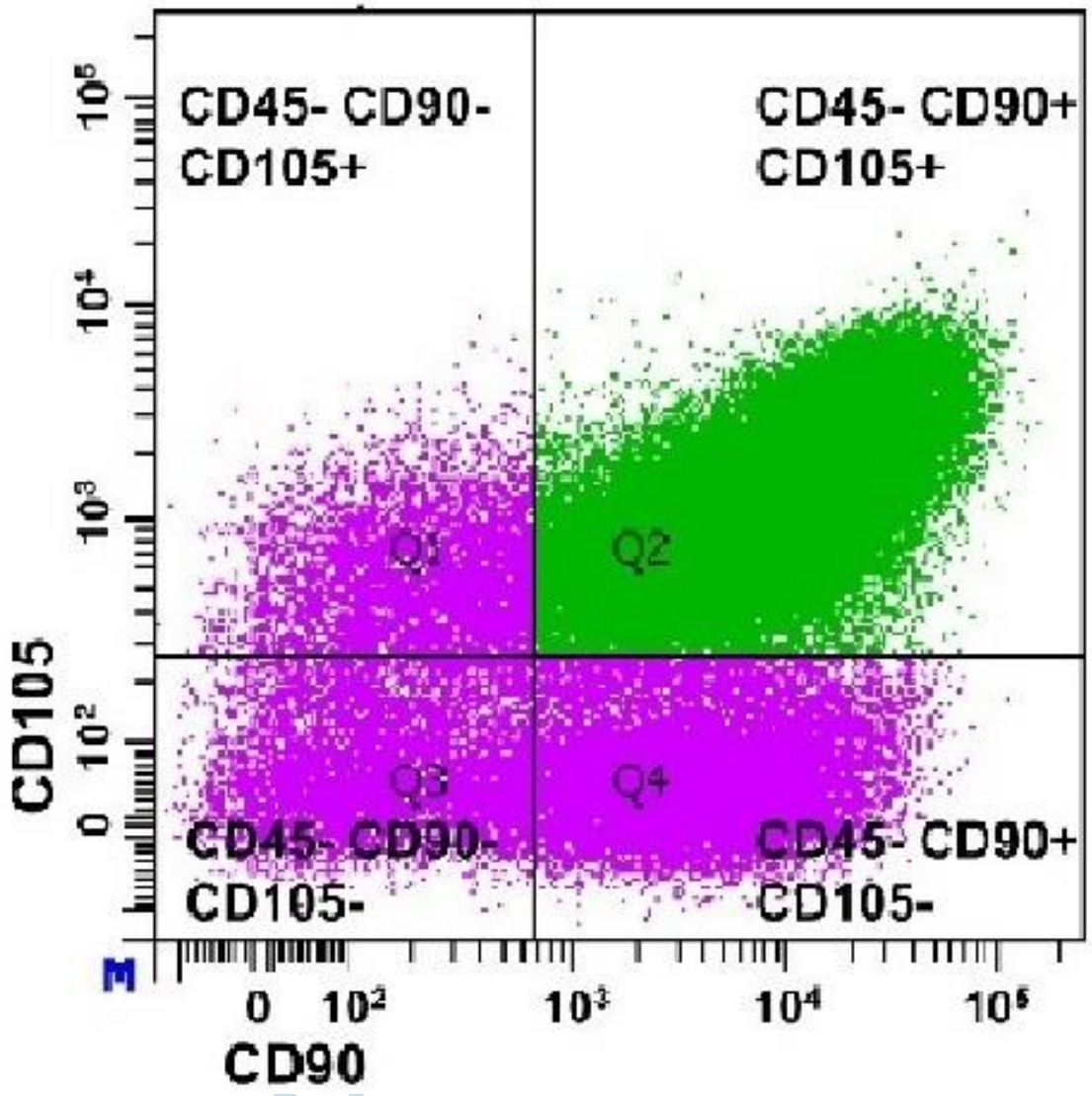

Figure 2E.

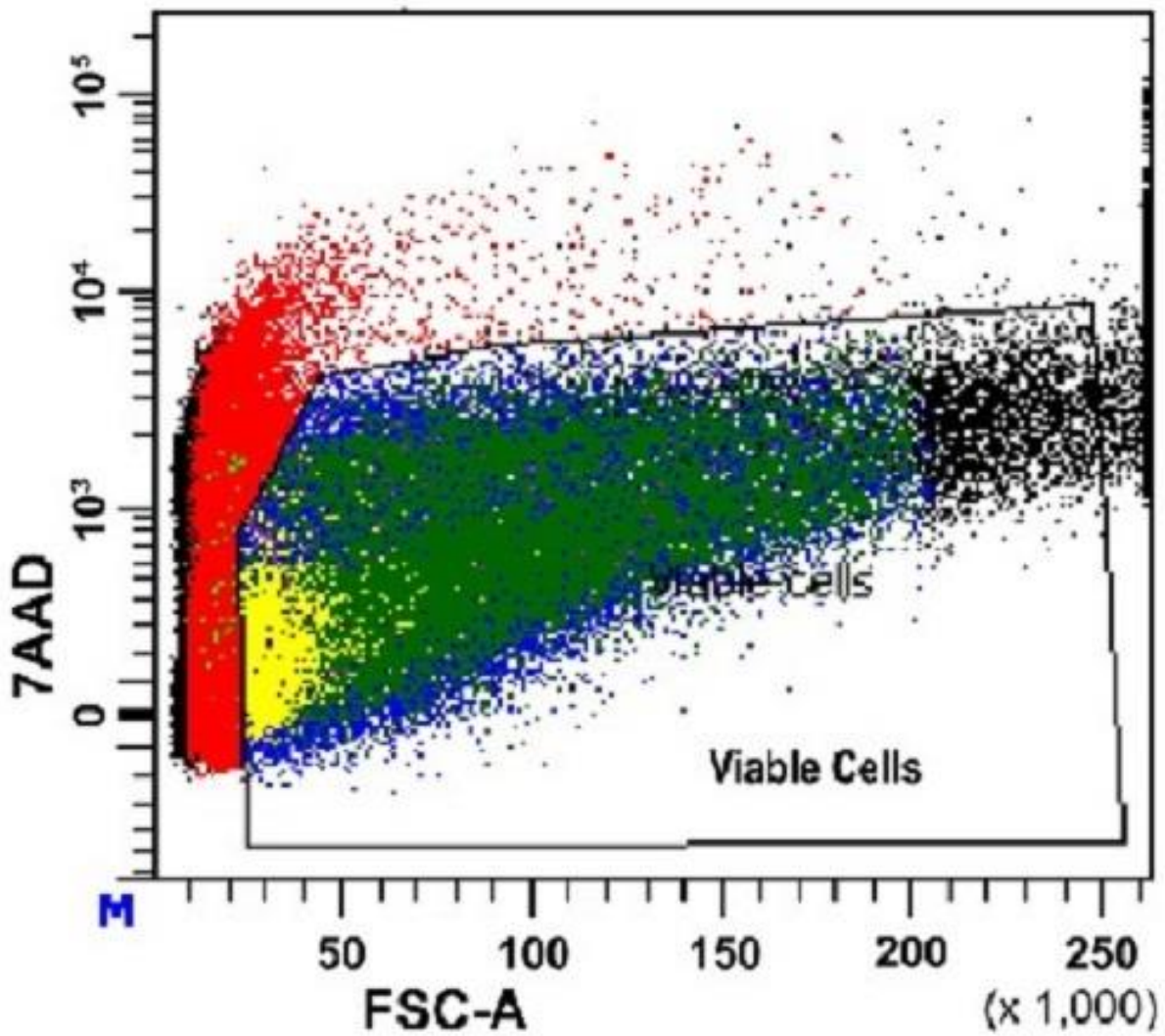

Figure 2F.

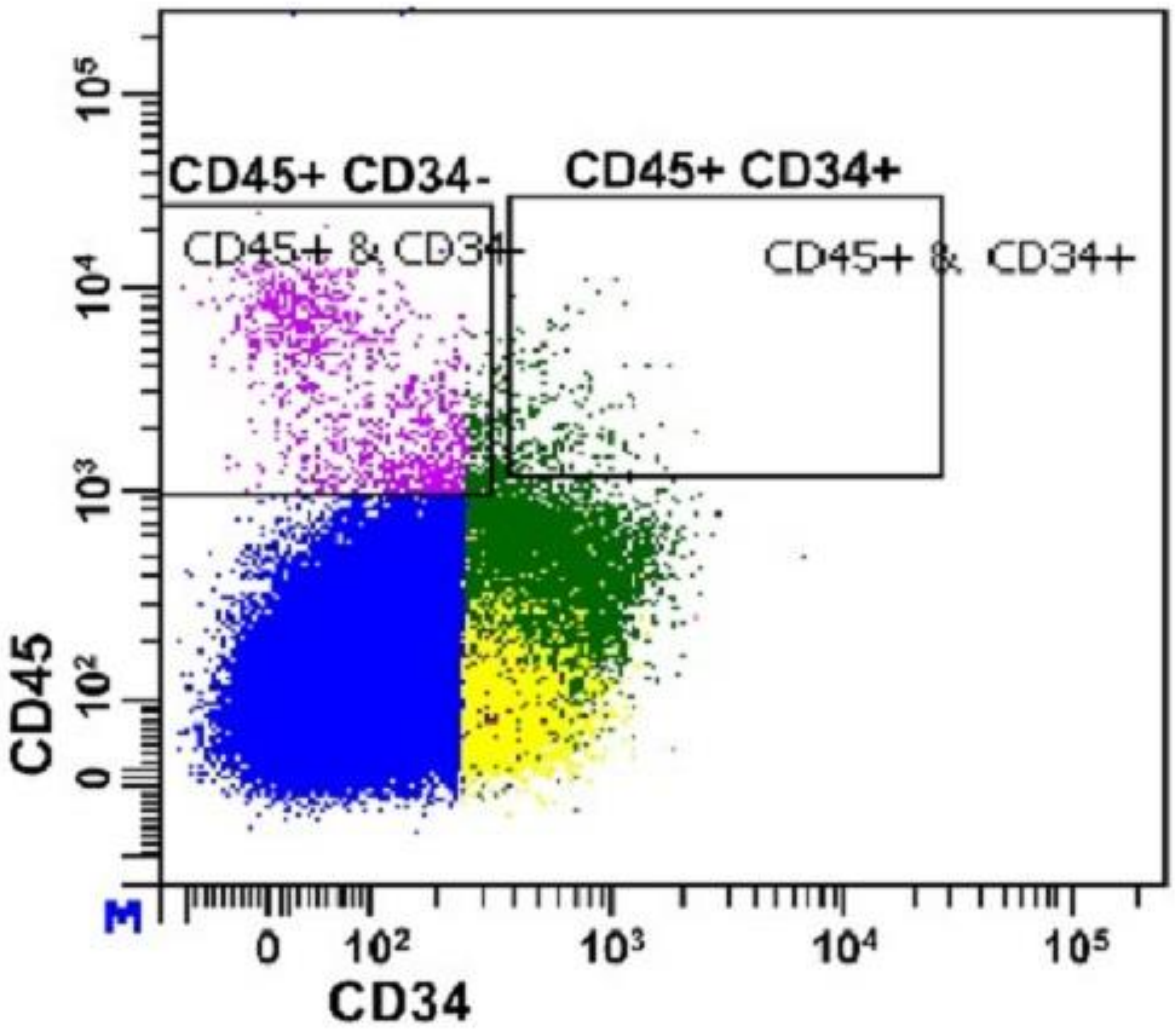

Figure 2G.

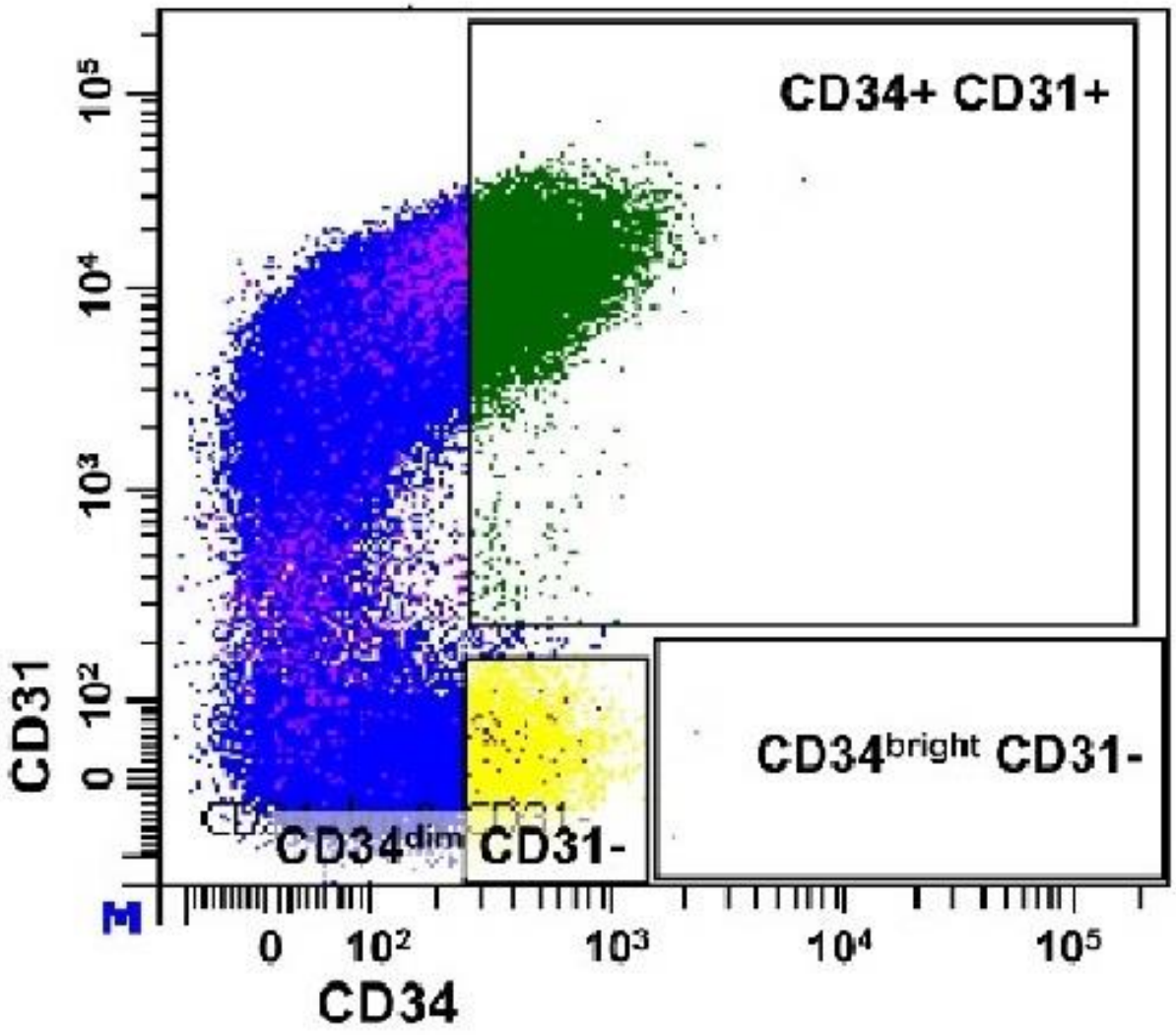

Figure 2H.

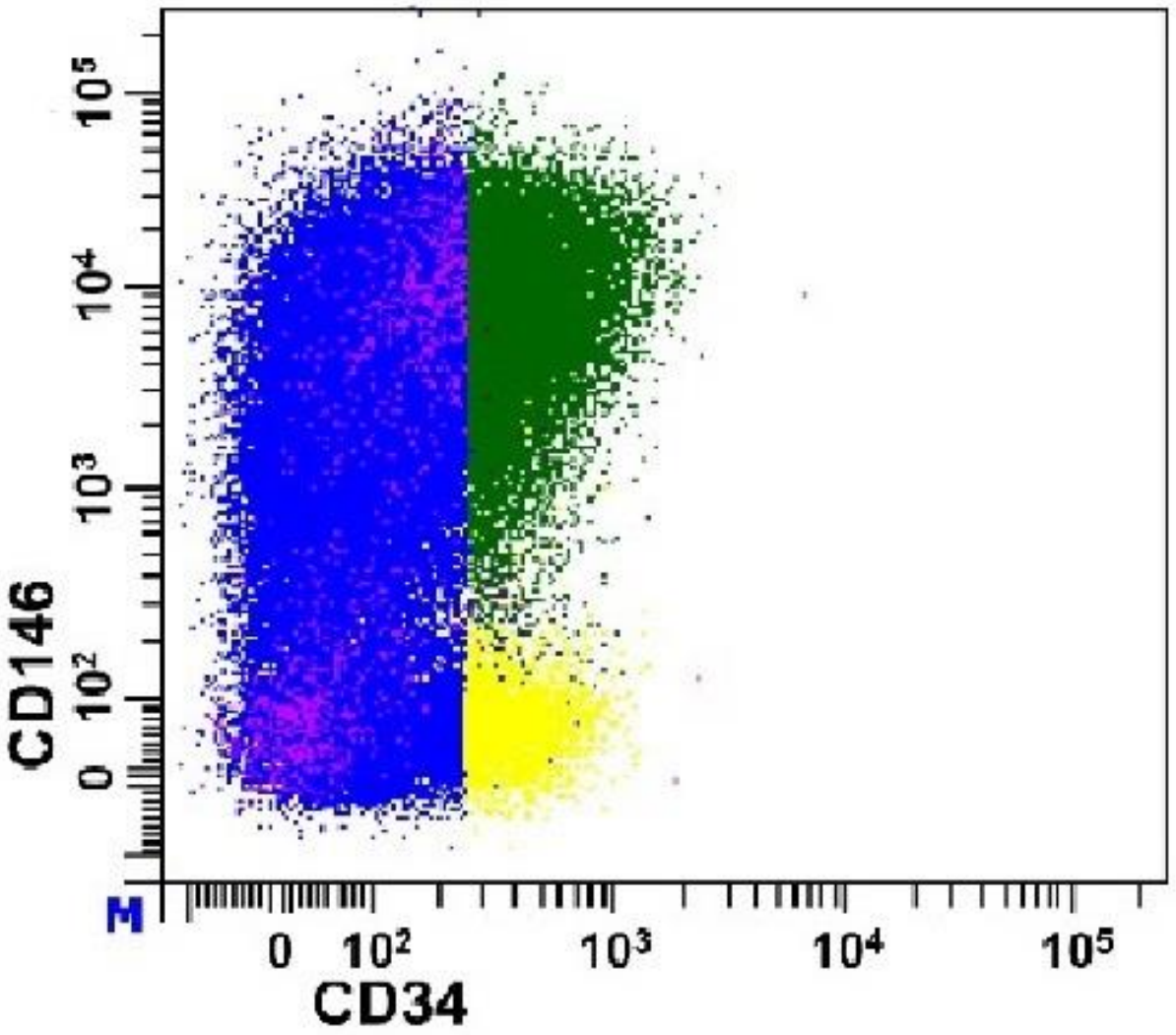

Figure 2I.

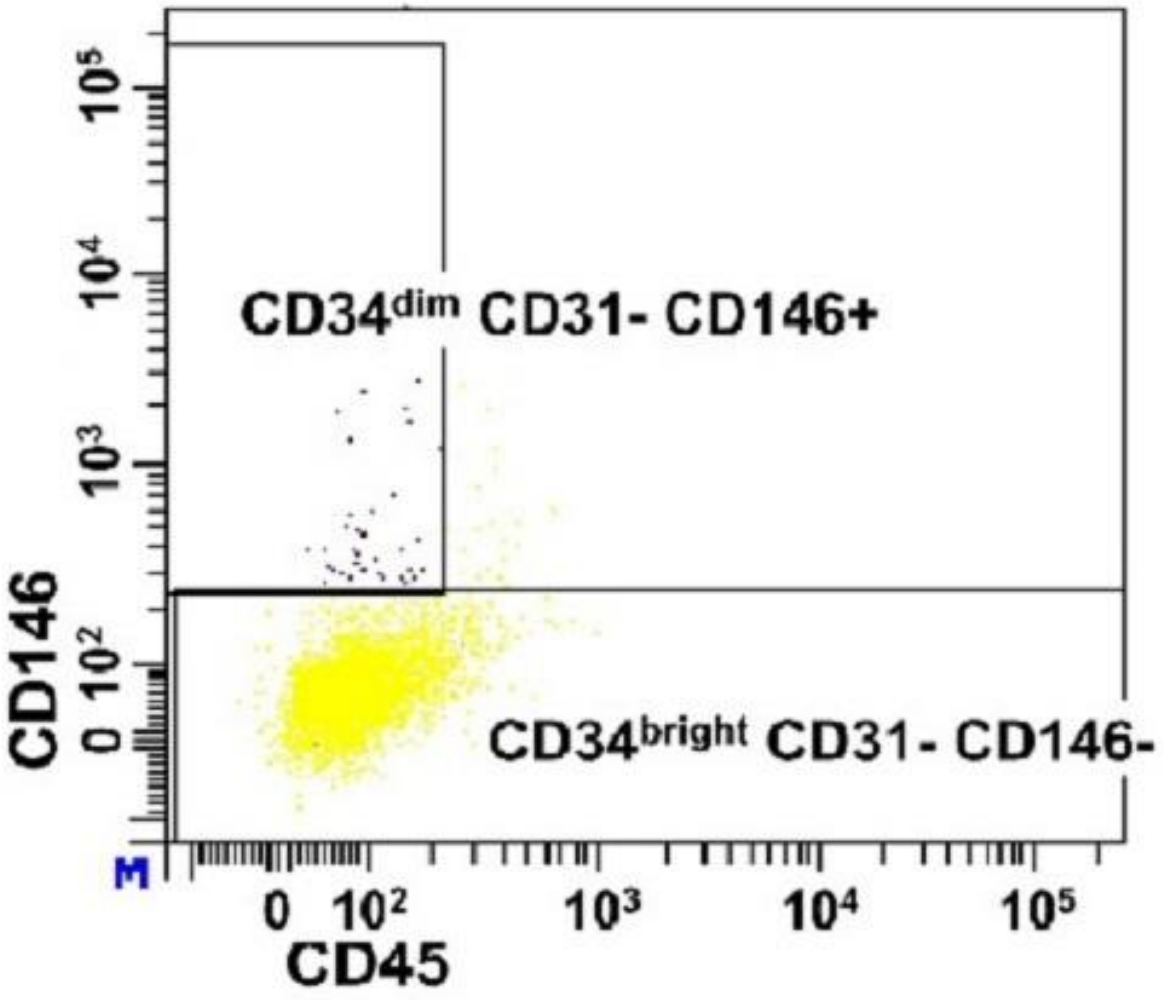

Figure 2J.

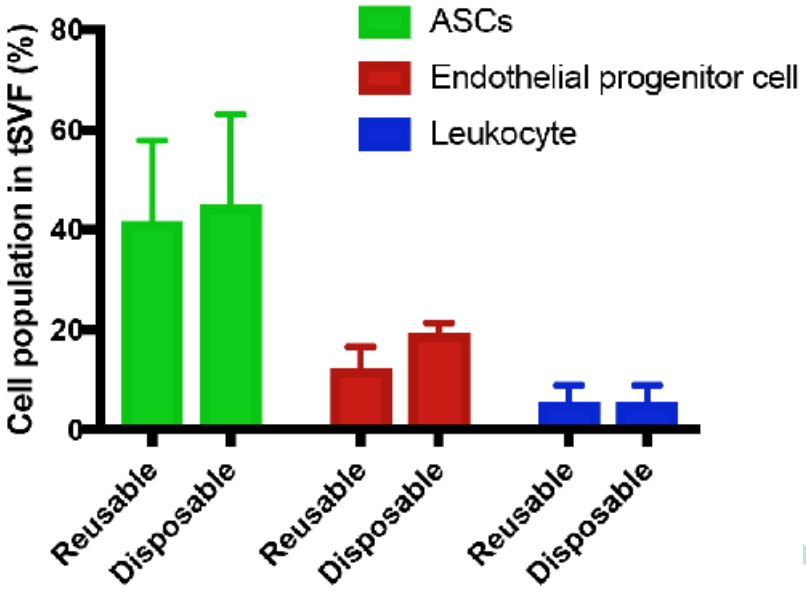

Figure 2K.

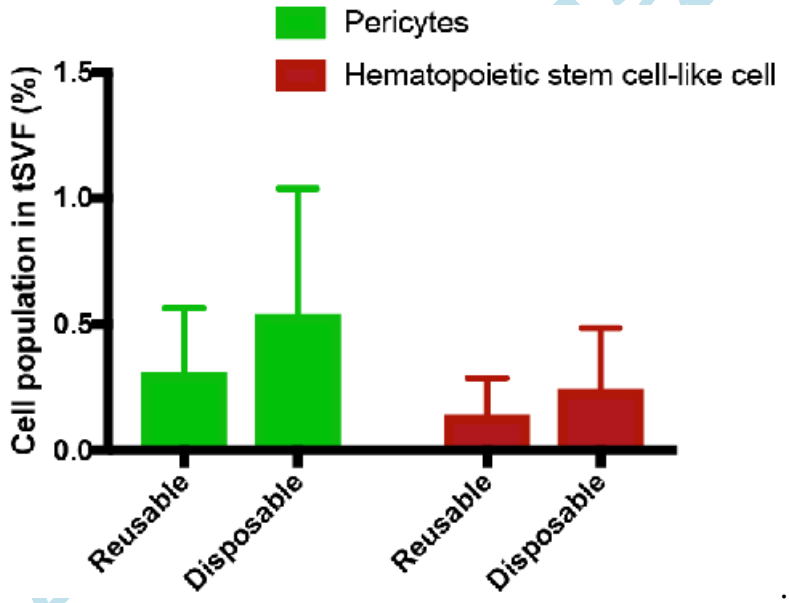

Figure 3.